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Chemical composition, antioxidant and antimicrobial Activity of the essential oil from the leaves of *Cymbopogon citratus*

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Abstract
Essential oils also known as volatile oils are important components of cosmetics. Recently cosmeceuticals are becoming popular throughout the world because cosmetics have therapeutic effect. This study investigated the chemical composition, antimicrobial and antioxidant activities of essential oil extracted from leaves of *Cymbopogon citratus, a* medicinal plant used in cosmetics. The essential oil of air-dried leaves of *Cymbopogon citratus* obtained through hydro distillation using Clevenger apparatus was analyzed by gas chromatography-mass spectrometry (GC-MS). The oil yielded 0.8% volume per dry weight of sample. The oil was composed majorly of sesquiterpenoids and diterpenoids. The major components of the essential oil of *C. citratus* were Ethyl elaidate (32.46%), Palmitic acid ethyl ester (25.64%) and Linoleic acid ethyl ester (21.02%).The antimicrobial assay was carried out using the agar diffusion method while the antioxidant assay was carried out using the1,1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant assay, ferric reducing antioxidant power, nitric oxide radical scavenging, total antioxidant capacity and lipid peroxidation assays on the oils. Results of the antioxidant activities of the essential oil of *C. citratus* showed that the oil has promising antioxidant potential. The oil was active against *Staphylococcus aureus* (ATCC29213), *Streptococcus mutans*, *Escherichia coli* (ATCC25922) and *Candida albicans* (ATCC10231), which are all microbes responsible for skin infections in humans. This reaffirms the use of this plant in cosmetics to fight skin infections and as preservatives.

Keywords: *Cymbopogon citratus*, cosmetics, essential oil, GC-MS, antimicrobial, antioxidants

1. Introduction
Cymbopogon citratus is grown as an ornamental plant and is used as a culinary and medicinal herb, the essential oil extracted from its leaves is widely known [1]. The compounds identified in *Cymbopogon citratus* are mainly terpenes, alcohols, ketones, aldehyde and esters. Some of the reported phytoconstituents are citral α, citral β, nerol geraniol, citronellal, terpinolene, geranyl acetate, Myrecene and terpinol methyl heptenone. The plant also contains reported phytoconstituents such as flavonoids and phenolic compounds, which consist of luteolin, quercetin, kaempferol and apiginin [2]. Lemongrass also contains z-citral, borneol, estragole, methyleugenol, geranyl acetate (3, 7-dimethyl-2,6-octadiene-1-ol acetate), geraniol (some species higher in this compound than citral), β-myrcene, limonenepiperitone, citronellal, citral-2, α-terpineole, pinene, farnesol, proximadiol and (+)- cymbodiacetal [3]. It was also reported that geranial, neral and geraniol are found in lemon grass and are attractive essential oils with potential medicinal applications [4]. The major constituents of lemongrass essential oil are neral (31.5%), citral (26.1%), and geranyl acetate (2.27%) [5]. In another work, the main constituents were geranial (33.7%), neral (26.5%) and myrcene (25.3%). Small amounts of neomenthol (3.3%), linalyl acetate (2.3%), Z-β-ocimene (1.0%) and E-β-ocimene were also detected [6]. Studies indicate that *Cymbopogon citratus* possesses various pharmacological activities such as anti-amoebic, antibacterial, antidiarrheal, antifilarial, antifungal and anti-inflammatory properties. *Cymbopogon citratus* essential oil is used in aromatherapy [2]. Lemongrass is antifungal and antibacterial in nature owing to citral, an organic compound that is found in its leaf. It is responsible for keeping bacteria and infections at bay, which means, when used on skin, it helps ward off acne, pimples and rashes. Lemongrass antioxidant-rich compounds are one of the factors that make it anti-inflammatory in nature, which is of great help to soothe irritated skin and calm redness, it is widely used as a toner to reduce the appearance of pores [7].

Nowadays much attention has been focused on essential oils that demonstrate antimicrobial activities and have been proposed as natural preservatives, antimicrobial activities of Essential oils *Cymbopogon citratus* are well known and documented in numerous works [8-9].

The DPPH, reducing activity and Nitrogen oxide assays of the antioxidant activities of essential oil of lemongrass leaves from North Indian plain clearly indicated that lemon grass essential oil is effective in scavenging free radical and has the potential to be powerful antioxidant [10]. The essential oil of *C. citratus* possess free radical scavenging activity which makes them potent natural antioxidants andis used as a natural remedy to heal wounds, help prevent infection and fight dandruff [11-12]. This research analyzed the chemical composition, antimicrobial and antioxidant activities of the essential oil of *Cymbopogon citratus* leaves and gave detailed information on the benefits that will be derived from incorporating the essential oil in cosmetics as natural preservative because of its antioxidant, antimicrobial activity and its availability.

2. Experimental

2.1 Plant material and essential oil extraction technique.

The healthy leaves of *Cymbopogon citratus* (Lemongrass) were collected from Olumo Igbogbo, Ikorodu, Lagos State, Nigeria in June, 2021. The botanical identification and authentication was done by Dr. Nodza George at the Herbarium in Botany Department, University of Lagos, Nigeria with authentication number LUH: 8801. The fresh leaves of *Cymbopogon citratus* were air-dried for four days and pulverized using mechanical grinder prior to extraction. The essential oil was obtained by hydrodistillation using 300g of the pulverized leaves using the Clevenger apparatus in 4 hours [13]. The oil obtained was dried over anhydrous sodium sulphate and stored in vials in a refrigerator prior to analysis.

2.2 GC-MS Analysis of Volatile oil

The analysis of the essential oil of *Cymbopogon citratus* was carried out using an Agilent 7820A gas chromatograph coupled to 5975C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies). The stationary phase of separation of the compounds was HP-5MS capillary column coated with 5% phenyl methyl siloxane (30m length x 0.32mm diameter x 0.25µm film thickness) (Agilent Technologies). The carrier gas was helium and it was used at constant flow of 1.4871 mL/min and at an initial nominal pressure of 1.4902 psi and average velocity of 44.22 cm/sec. 1µL of the samples were injected in splitless mode at an injection temperature of 300 °C. Purge flow to spilt vent was 15 mL/min at 0.75 min with a total flow of 16.654 mL/min; gas saver mode was switched off. Oven was initially programmed at 40 °C for (1 min) then ramped at 12 °C/min to 300 °C (10 min). Run time was 32.667 min. with a 5 min. solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C and transfer line temperature of 280 °C. Acquisition of ion was via Scan mode (scanning from m/z 45 to 550 amu at 2.0s/scan rate).

Relative percentage amounts of the essential oil components were evaluated from the total peak area by apparatus software. Identification of components in the volatile oil was based on the comparison of their mass spectra and retention time with literature data and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

2.3 Antioxidant Assay

2.3.1 DPPH Free Radical Scavenging Assay

The free radical scavenging capacity of the essential oil of *Cymbopogon citratus* was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method [14]. A solution of 0.1mM DPPH in ethanol was prepared, 1mL of the solution was added to 1 mL of the essential oils at different concentrations (25, 50,75, 100 µg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Ascorbic acid (4 mg/mL in ethanol) was used as positive control while ethanol was used as negative control. Then the absorbance was measured at 517 nm by using Ultraviolet Visible Spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH scavenging effect was calculated by plotting inhibition % against concentration to obtain the IC_{50}. The test was repeated for all concentrations used in triplicates.

2.3.2 Ferric reducing antioxidant power assay (FRAP)

In the FRAP assay, the method of Pham-Huy *et al.* [14] was adopted, the absorbance at 700 nm was measured using Ultraviolet Visible Spectrophotometer against a blank. Gallic acid was used as the control. A higher absorbance of reaction mixture indicated greater reducing power. Data was presented as mean and standard deviation for triplicate analysis. The percentage FRAP Scavenging effect was calculated as described for the DPPH assay.

2.3.3 Nitric oxide radical scavenging assay.

The method described by Okoh *et al.* [15] was adopted. Nitric oxides radicals were generated from a sodium nitroprusside solution; Sodium nitroprusside (1 mL of 10 mM) was mixed with 1 mL of oil to give concentrations of 0.025–0.50 mg/mL in phosphate buffer. The mixture was incubated at 25 °C for 150 min. To 1 mL of the incubated solution, 1 mL of Griess' reagent was added. The absorbance was measured at 546 nm using a UV/VIS TG 50 Plus UV-Vis microplate reader (Molecular Devices, GA, USA). Ascorbic acid was used as the positive control. The % inhibition of nitric oxide radical by the oil was calculated as described for the DPPH assay.

2.3.4 Lipid peroxidation

In this assay, 10 µL of essential oil at different concentrations of 25,50,75 and 100 µg/mL or standard solution, (1,1,3,3-tetramethoxypropane, TEP) and 40 µL of 20 mM phosphate buffer (pH 7.0) were added to test tubes on ice bath. In each tube, 50 µL of 3% sodium dodecyl sulfate (SDS), 200 µL of 0.1 N HCl, 30 µL of 10% phosphotungstic acid, and 100 µL of 0.7% of 2-thiobarbituric acid (TBA) were added. The tubes were firmly closed and boiled at 100°C for 30 min in water bath. The reaction mixture was mixed with 400 µL of n-butanol and then centrifuged at 3000 rpm for 10 min. Ascorbic acid was used as positive control. Supernatants were collected and pass through Ultraviolet Visible Spectrophotometer at a wavelengths of 515 nm/555 nm [16]. The percentage of inhibition of lipid peroxide was calculated using the equation described for the DPPH assay.

2.4 Total antioxidant capacity

The method described by Kattamis *et al.* [28] was adopted and Ultraviolet Visible Spectrophotometer was used to determine the absorbance of the mixture (optical density, OD, of 570

nm). Ascorbic acid was used as control. The percentage of total antioxidant capacity was calculated using the equation described for the DPPH assay.

2.5 Antimicrobial Assay

The antimicrobial activity of essential oil of *Cymbopogon citratus* was assayed using Agar well diffusion technique [18]. The inocula were prepared from the typed bacteria and yeast cultures of *Staphylococcus aureus* (ATCC 29213), *Streptococcus mutans* (clinical isolate), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231) respectively which were maintained in glycerol-peptone water at 4ºC in the pure culture laboratory of Microbiology Department, University of Lagos, Akoka-Yaba, Lagos, Nigeria and were sub-cultured into sterile peptone water in McCartney bottles. The densities of the bacterial suspensions were determined by diluting the broth cultures 1:100 (mixing 0.1mL of the inoculum and 9.9ml of sterile normal saline). These were compared with 0.5 McFarland standards. These suspensions were estimated to 1.0 x106 - 107 CFU/Ml. Standard broad spectrum antibiotic discs -Ciprofloxacin and Pefloxacin (10µg –Maxicare Med. Lab, Nig) were used as positive control while hexane served as negative control.

3. Results and Discussion

3.1 Chemical composition

The yield of *Cymbopogon citratus* essential oil obtained by hydrodistillation was 0.8% v/w. The chemical analysis by Gas Chromatography-Mass Spectrometry (GC-MS) identified 31 compounds.

Table 1: Chemical Composition of the Essential Oil from *Cymbopogon citratus*

Compound	% Composition	RI-calc	RI-lit
Octane	0.09	819	816
cis-Sabinol	0.08	1081	1085
α-N-Methyl ionone	0.11	1433	1429
trans-Nerolidol	0.9	1566	1564
Lauric acid, ethyl ester	0.1	1584	1580
Myristic acid, ethyl ester	0.78	1776	1779
Myristic acid, isopropyl ester	0.08	1815	1814
Octadecanoic acid, ethyl ester	0.07	2169	2167
Chloromethyl 7-chlorododecanoate	0.13	1651	1648
Hexadecanoic acid, methyl ester	1.68	1880	1878
Butyl tetratriacontyl ether	-	3877	3874
1-Tetradecanol	0.14	1658	1656
Pentadecanoic acid	2.89	1866	1869
Ethyl 9-hexadecenoate	1.82	1988	1986
Palmitic acid, ethyl ester	25.64	3739	3735
Palmitic acid	0.29	1971	1968
Ethyl ricinoleate	0.09	2346	2347
Propyl palmitate	0.38	2074	2077
9,12-Octadecadienoic acid, methyl ester	1.52	2373	2377
Oleic acid, methyl ester	2.23	2090	2085
Methyl stearate	0.19	2129	2133
Linoleic acid	1	2180	2183
Oleic Acid	2.73	2178	2175
Citral	53.48	1170	1174
Stearic acid, ethyl ester	2.46	2185	2181
Isopentyl decanoate	0.11	1620	1615
n-Propyl 9, 12-octadecadienoate	0.25	2294	2292
trans-9-Octadecenoic acid, pentyl ester	0.43	2175	2175
cis-Vaccenic acid	0.08	2118	2116
Ethanol, 2-(tetradecyloxy)-	0.1	1930	1930
Pentyl tetradecyl ether	0.15	1986	1986

Notes: RI-calc, retention index calculated on HP-5MS column relative to C8–C20 n-alkanes; RI-lit, retention index reported in the literature

Table 2: Antimicrobial activity of *Cymbopogon citratus* essential oil using the disc diffusion method

Microorganisms	Zones of inhibition		
	CcCPX	PEF	
Staphylococcus aureus	30.00±0.7	29.00±0.7	25.00±0.0
Escherichia coli	39.00±0.0	27.00±0.0	26.00±0.7
Streptococcus mutans	38.00±0.0	27.00±0.7	27.00±0.0
Candidaalbicans	38.00±1.4	29.00±0.0	26.00±0.0

Abbreviations: Cc, *Cymbopogon citratus;* CPX, Ciprofloxacin; PEF, Pefloxacin. Results are means of triplicate values.

Table 3: Antioxidant activity of *Cymbopogon citratus* essential oil.

Antioxidant assays	Ic_{50} µg/mL -Cc	Ic_{50} µg/mL (Ascorbic acid and Gallic acid)
DPPH (%Inhibition)	71.82	29.61
FRAP (%Inhibition)	10208.16	8466.44
NO (%Inhibition)	24.9	34.24
LP (%Inhibition)	94.25	34.24

TAC (mg/100)	39.15	
Total flavonoids (mg/100)	46.92	
Total phenol (mg/100)	34.37	

Antioxidant activities - DPPH, 1, 1-diphenyl-2-picrylhydrazyl; FRAP, Ferric reducing antioxidant power; NO, Nitric oxide; LP, Lipid peroxidation
TAC, Total antioxidant activity
Cc, *Cymbopogon citratus*; Control: Ascorbic acid; Gallic acid

The *Cymbopogon citratus* essential oil component as shown in Table 1 contain many aliphatic esters, with few acids and ketones. However, the major components of the oil are a monoterpenoid, citral (53.48%) and an aliphatic ester, palmitic acid ethyl ester (25.64%). The monotepenoids, sabinol, methyl ionone and nerolidol were also present in minute quantities. Several other studies reported that oxygenated monoterpenoids are the major constituents of lemongrass essential oil these include citral (Neral and geranial), geraniol, geranyl acetate, citronellal, citronellol, 1, 8-cineole (eucalyptol), α-terpineol, linalool and elemol [2, 5, 19, 20, 21, 22, 23]. The presence of aliphatic esters is a variation observed in essential oil studied and this may be due to geographical location and age of the plant parts extracted among other factors [24].

3.2 Antimicrobial Activity

The antimicrobial potential of *C. citratus* essential oil investigated using Agar well diffusion technique exhibited significant *in vitro* antimicrobial activity against *Staphylococcus aureus* (30.00±0.7), *Streptococcus mutans* (38.00±0.0), *Escherichia coli* (39.00±0.0) and *Candida albicans* (38.00±1.4) (Table 2). The results compare well with that of the standard drugs (25.00-29.00). The promising antimicrobial activity of the essential oil could be attributed to the presence of citral in the oil [25, 26]. This indicate that lemon grass oil,if incorporated into cosmetics can be used tomanage and treat various skin and soft tissue infections, abscesses (boils), furuncles etc. Almeida *et al*. [8] reported that the essential oil of *Cymbopogon citratus* showed microbiostatic and microbicidal activity against all tested strains of *Staphylococcus* spp., *Streptococcus mutans* and *Candida* spp. It was also reported that the essential oil of lemon grass was effective in controlling bacterial growth in biofilms of *Streptococcus mutans* [27]. This reaffirms the possible use of *Cymbopogon citratus* essential oil in cosmetics formulations to fight skin infections and as preservatives. *Cymbopogon citratus* is widely available to replace synthetic preservatives which often cause skin irritation and lead to allergenic reactions [9].

3.3 Antioxidant Activity

The antioxidant activity of the essential oil of *Cymbopogon citratus* was evaluated by measuring its radical scavenging activity using DPPH, Ferric reducing antioxidant power, Nitric oxide and Lipid peroxidation methods. The IC_{50} of DPPH (71.82 µg/mL), FRAP (10208.16 µg/mL), Nitric Oxide Scavenging activity (24.90 µg/mL) andLipid peroxidation scavenging activity (94.25 µg/mL) are presented in Table 3. Comparatively, strongernitric oxide scavenging activity was observed in the essential oil (IC_{50} of 24.90 µg/mL) compared to that of the standard drug (IC_{50} of 34.24 µg/mL), while weak antioxidant activity was observed in Lipid Peroxidation scavenging activity of the oil (IC_{50} of 94.25 µg/mL) compare to the standard drug used (IC_{50} of 34.24 µg/mL).The standard drug (Ascorbic acid) used demonstrated higher antioxidant activity for DPPH and Ferric reducing antioxidant power (FRAP) than the essential oil.

4. Conclusion

The essential oil of *Cymbopogon citratus* (lemon grass) was composed of manyaliphatic esters, but the main components are, citral and palmitic acid ethyl ester. The essential oil showed promising antimicrobial and antioxidant capacity, which is suggested to be due to the presence of the major component of the oil, citral. The results suggest the use of the essential oil of lemon grass in cosmetic products to fight skin infections and as preservatives.

5. Competing Interests

Authors have declared that no competing interest exist.

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